

BEETURIA: ITS INCIDENCE AND A CLUE TO ITS MECHANISM

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Beeturia is the pink to deep red coloration of the urine which occasionally follows the eating of beetroot. It is observed in only a small section of the population. Little is known about the phenomenon, but opinions vary from the unsubstantiated popular belief that it will occur in anyone who eats enough beetroot, to claims that it is only found in association with food allergy (Zindler and Colovos, 1950) or that its incidence is genetically determined (Allison and McWhirter, 1956).

For many years the red pigment from *Beta vulgaris* has erroneously been considered to be a nitrogenous anthocyanin (Meyer, 1960) and its urinary excretion described as anthocyaninuria. Recent investigations have shown that the pigment belongs to a new class of red-violet pigments called betacyanins, which although nitrogenous are only loosely related to the anthocyanins and appear to be more accurately described as a type of alkaloid (Mabry *et al.*, 1962). The correct clinical term, therefore, is betacyaninuria or, more simply, beeturia.

Our interest was aroused by a patient who presented with severe anaphylactic shock associated with gross beeturia, and an investigation was started in an attempt to explain the mechanism of betanin absorption. The animal experiments of Horwitt (1933) showed that anthocyanin from concord grapes was invariably excreted by the kidney after intravenous administration, but in the rat and dog this did not occur after feeding it. We have shown that the same is true for betanin in rats and also in the human subject (Watson, 1963), where at least 75% of the injected pigment has been recovered from the urine. Therefore the mechanism for beeturia seemed largely, if not wholly, to depend on absorption and not excretion or impaired inactivation of the pigment as stated by Allison and McWhirter (1956).

If the findings and assumptions of Zindler and Colovos (1950) were correct, there was a possibility that beeturia might be encountered in patients with gluten-induced enteropathy, or that its presence or absence in patients with the malabsorption syndrome might distinguish between those whose disorder could be related to beet allergy in particular, or to food allergy in general, and those with no such abnormality. The results point to different but equally interesting conclusions.

Methods

After some initial experimentation the following test was adopted: 100 g. of commercially prepared whole small beets was given without food at 9 p.m., and the subject's bladder emptied at this time. All urine excreted thereafter was collected until 8 a.m. next morning, and tested for pigment within three hours of the completion of the test. Standard tests for the identification of beet pigment include spectrophotometry, fluorescence, and solvent solubility properties. However, when beeturia is anticipated, the presence of a rose-pink colour in an acid urine, which disappears on alkalization and reappears after reacidification, is sufficient proof of its presence. At intervals absorption spectrophotometry of positive samples was carried out, and

curves were plotted and compared with those for urine to which solutions of beet pigment had been added. These tests always corroborated the side-room verdict.

This test utilizes an amount of beetroot which if not a "maximum test dose" is much more than anyone would eat from free choice. It was taken by all subjects, although with difficulty in a few cases.

In those studies involving the administration of iron this was given either orally as ferrous sulphate 200 mg. three times a day or intramuscularly as "imferon" 4 ml. daily to a calculated total dose.

Results

The numbers and types of subjects are shown in Table I, which includes the number of positive results for each group and their significance values, compared with the control group. The control group consisted of nurses, technical and medical staff, and dental and medical students

TABLE I.—Results of 178 Beetroot Tests (The Statistical Comparisons Relate to the Normal Group)

Clinical Group	No.	Positive	Incidence	χ^2	Significance
Normal ..	58	8	13.8%		
Miscellaneous illnesses ..	48	6	12.5%	0.034	P>0.50
" anaemias ..	7	1	14.3%	0.001	P=0.98
Pernicious anaemia ..	11	5	45.5%	6.1	P<0.02
Malabsorption syndrome ..	18	7	39.0%	5.5	P<0.02
Iron deficiency ..	35	17	48.6%	13.8	P<0.005

in good health and without a known food allergy. The miscellaneous group was made up of ward patients with a variety of clinical conditions, but excluding renal disease or any of the other conditions listed in Table I. Some patients with mixed anaemias—for example, iron-deficiency and pernicious anaemia—were studied, but are not included in the figures. The miscellaneous anaemias were "refractory" (two cases), haemolytic (one case), and anaemia in association with subacute bacterial endocarditis, colonic cancer, acute disseminated lupus erythematosus, and chronic myeloid leukaemia (one case each). The malabsorption group is a heterogeneous group of patients with diverse alimentary disorders, including post-gastrectomy steatorrhoea, duodenal carcinoma, chronic pancreatitis, tuberculous enteritis, and idiopathic steatorrhoea.

The results for the normal and miscellaneous groups are very close and there is clearly no difference between them. This applies also to the small group of miscellaneous anaemias, whose average haemoglobin was 69% (range 58–78%). The 45.5% incidence of positives in the pernicious-anaemia group is significantly higher than normal and will be considered in the general discussion.

The groups which remain for consideration are the most interesting.

The heterogeneity of the malabsorption group obscures the significance of the seven positive results, for not only is the group a mixed one in terms of pathology but the clinical features involve wide ranges of malabsorption defect in terms of faecal-fat excretion and xylose absorption, as well as variable degrees and types of anaemia.

However, the group contains a subgroup of 11 patients in whom jejunal biopsy by Crosby capsule showed total or subtotal villous atrophy. Of these 11 patients, five had beeturia ($\chi^2=6.1$, $P<0.02$), two of the results being the most strongly positive of the entire series and approaching the intensity of the patient we had seen with anaphylaxis. These two had no symptoms during the test attributable to beetroot. Even the property of jejunal atrophy does not make these five homogeneous in terms of a possible cause for the beeturia, for one had a severe megaloblastic anaemia (Hb 35%) and another a severe iron-deficiency and megaloblastic anaemia (Hb 30%) at the time of the test. If these two are excluded from the analysis of the figures there are three positive beetroot tests in nine patients with jejunal atrophy, an incidence which is not significant compared with the control group ($\chi^2=2.1$, $P>0.10$).

The iron-deficiency group was built up deliberately as the study progressed, because early results suggested that the iron-deficient state as well as, if not rather than, intestinal disease was involved in the phenomenon of beeturia. The causes of the iron-deficiency anaemias were menorrhagia, haemorrhoids, bleeding peptic ulcers, hiatus hernia, and poor diet.

The 17 positive results in this group of 35 patients is a significantly greater incidence than in the control group ($P<0.005$). The results were examined from many aspects, and Table II summarizes the information about the patients which seems to be relevant. The principal fact revealed by this presentation of the data is the influence of orally administered iron on the result of a test. If the results are compared on the basis of "given iron" or "not previously given iron" (Table III) there are 16 positive tests in 20 patients not previously given iron (80%) and only one positive in 15 being given iron (6.7%). The difference between the groups is significant ($\chi^2=18.3$, $P<0.001$).

This apparently clear division of the results is complicated slightly by the fact that the difference of 9.9% between the average haemoglobin levels of the positive and negative groups is also significant ($t=2.64$, $P<0.02$). However, if 16 subjects in Table II, indicated by an asterisk, are matched on the basis of their haemoglobin levels, two

TABLE II.—Results of Beetroot Tests on 35 Patients with Iron-deficiency Anaemia Related to Haemoglobin Level and Administration of Oral Iron (100% Haemoglobin=14.8 g./100 ml.)

Test Positive (17)			Test Negative (18)		
Haemoglobin (%)	Oral Iron		Haemoglobin (%)	Oral Iron	
	Yes	No		Yes	No
26		+	41		+
34		+	*49	+	
45		+	54		+
45		+	*59	+	
48		+	*59	+	
48		+	60		+
49		+	*65	+	
*50		+	*65	+	
51		+	65	+	
51		+	68	+	
*58	+		69	+	
*59		+	*70		+
*62		+	70	+	
*65		+	71	+	
*70		+	71	+	
*72		+	*72	+	
*74		+	*75	+	
Av. 53.4	1	16	Av. 63.3	14	4

* 16 subjects matched on the basis of their haemoglobin levels.

TABLE III.—Beetroot Tests in Iron-deficiency Group (35) (The Statistical Comparisons Relate to the Normal Group)

	No.	Positive	Incidence	χ^2	Significance
Given oral iron	15	1	6.7%	0.62	$P>0.1$
No iron	20	16	80.0%	29.6	$P<0.001$

groups of eight are obtained whose mean haemoglobin levels are 63.8% and 64.3% respectively. This now gives seven positive results in a group of eight "not previously given iron" and seven negative results in a group of eight "given iron." The difference between these results is still significant ($\chi^2=9.0$, $P<0.005$).

Proof of the dependence of the result of the test on whether or not the subject has been given oral iron does not rest exclusively on this statistical manipulation. Table IV gives the results of tests on seven patients (five with iron-deficiency anaemia, two with pernicious anaemia) carried out before they had received oral iron and while they were receiving it during a period when the haemo-

TABLE IV.—Results of Beetroot Tests in Seven Anaemic Patients Before the Administration of Iron and on Fourth and Eighth Days of a Course of Iron Therapy

Patient	Anaemia	Result			Route of Administration
		Initial	After 4 Days	After 8 Days	
1	I.D.	++ (2)	+	—	Orally
2	"	++	+	—	"
3	"	+	Trace	—	"
4	"	++ (2)	+	Trace	I.M.
5	P.A.	+	+	Trace	Orally
6	P.A.	++ (3)	+	"	I.M.
7	P.A.	++ (2)	+	"	I.M.

I.D.=Iron deficiency. P.A.=Pernicious anaemia. Figures in parentheses show the number of times a test was performed before giving iron.

globin level rose by only a few per cent. A semiquantitative assessment of the degree of beeturia was used in this group of tests, and is graded as 2+, 1+, trace, or absent. There seems little doubt that it is the giving of iron rather than any marginal rise in haemoglobin which alters the nature of the result. We also suspect that oral iron is more effective in suppressing beeturia than parenteral iron, although there is no proof of this at present.

Discussion

This investigation shows that only a small number of normal people given a large helping of beetroot will exhibit beeturia. The incidence of 13.8% in the control group is duplicated by the miscellaneous-illness group, and compares closely with the incidence of 12.6% in 78 patients tested by Zindler and Colovos (1950) who were under investigation for, but not shown to have, a food allergy. We are certain that differences in results are not due to batch variations in beetroot manufacture; positive and negative results were obtained from test doses prepared from the same jar. The permissive mechanism of beeturia must lie either in the absorption of the pigment or in the inhibition of its destruction or denaturation should its absorption be universal. There is certainly no renal block to its excretion (Watson, 1963).

We believe that the problem is one of absorption and not denaturation, and this study suggests that the absorptive mechanism is influenced by the oral administration of iron. The incidence of beeturia is high in iron-deficient subjects and is highest of all in such patients who have not received oral iron. It is not affected by anaemia *per se*, and is indeed lowest in anaemic subjects who have received a course of oral iron. The facts suggest that beeturia is more likely to occur at a time of "iron hunger," perhaps via the pathway for iron absorption, and that there is competition between iron and betanin for the acceptor substance (could it be apoferritin?), with iron in a strongly preferential position. Iron-balance studies using ^{59}Fe , carried out in one of the cases of pernicious anaemia with a positive beetroot test, showed absorption of 60% of the dose. It is this

state of augmented iron absorption, known to occur during the treatment of pernicious anaemia with vitamin B₁₂ (Pirzio-Biroli *et al.*, 1958), which we feel might explain the high incidence of beeturia in the pernicious-anaemia patients.

Some of the 14% incidence of beeturia in normal subjects could be attributed to the fluctuating nature of iron absorption in normal people. In this respect it may be relevant that when some of the positives in the control group were retested the result was negative. Moore (1955) stresses that the regulation of iron absorption by the gut is not absolutely related to tissue needs. Clearly the problem is much more complex than the simple genetic hypothesis of Allison and McWhirter (1956), whose results were variable, whose conclusions have been challenged (Penrose, 1957), and whose views, even if correct, relate only to the incidence of the phenomenon in normal people and not to its mechanism. Other theoretical considerations which might be relevant in relating iron to the transport of betacyanin include the effect of iron deficiency on the function of cytochrome enzymes, or the reciprocal chelating properties of iron and betacyanin.

Experimental studies have been carried out to test other factors which might influence betanin absorption. Full body-weight doses of histamine, large doses of ascorbic acid, and 0.1N hydrochloric acid have not significantly affected repeat tests which were negative first time. The giving of food introduces so many variables that we have avoided this at present.

The possibility of a relationship between idiopathic enteropathy and beeturia is still undecided. The numbers do not permit statistical proof of an association, but the incidence of three positive tests in nine patients with

jejunal atrophy and no significant anaemia is suspiciously high, considering how strongly positive two of the tests were.

Further work on the metabolism of betanin is in progress, and we hope in the future to be able to give a more definitive account of this rather neglected clinical phenomenon.

Summary

Beeturia occurs in 13.8% of the normal population. It is not a universal phenomenon as is popularly supposed, nor is it limited to patients with food allergy as others claim.

The incidence of beeturia in patients with iron-deficiency anaemia is 48.6% and in iron-deficient subjects not previously treated with oral iron it is 80%. The influence of oral iron therapy on the phenomenon suggests that the apoferritin carrier mechanism may play some part in its occurrence.

There is an unsubstantiated possibility that beeturia may occur in certain kinds of "idiopathic enteropathy."

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BLOOD PYRUVIC ACID IN THALIDOMIDE NEUROPATHY

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Prior to the recognition of the teratogenic properties of thalidomide, numerous cases of peripheral neuropathy had been reported in adults after its use (Fullerton and Kremer, 1961; Burley, 1961). But its mode of action in causing the peripheral neuropathy was uncertain. Antagonism of the activity of vitamins of the B-complex was suggested by Robertson (1962), whilst an impairment of glucose absorption was reported by Meade and Rosalki (1961), although Kremer and Fullerton (1961) could find little evidence for this. Furthermore, any interference of glucose metabolism seemed unlikely in view of the finding of normal quantities of pyruvic acid in the blood (Fullerton and Kremer, 1961).

However, the standard methods in current use for estimating pyruvic acid, such as that of Friedemann and Haugen (1943), are unsatisfactory in so far as they are not specific and measure in addition other α -keto acids present in the blood; indeed, pyruvic acid may account for only 40-75% of the total (Cavallini, Frontali, and Toschi, 1949; El Hawary and Thompson, 1953), so that minor variations in its concentration may not be detected. Chromatography of the phenylhydrazine derivatives of keto acids (Cavallini *et al.*, 1949) allows of the separation of pyruvic acid from the other keto acids and enables its accurate determination (El Hawary and Thompson, 1953), so that lesser degrees of abnormality of pyruvate metabolism may thereby be detected (McArdle, 1957). This paper describes the results

of estimations of blood pyruvic acid by chromatography in three patients with thalidomide neuropathy.

Case 1

A retired nurse aged 66 presented with a 10-months history of aching pains in the feet, which progressively became worse and spread to involve the thighs. She experienced difficulty in walking owing to dead feelings in the legs and noticed stiffness and slight swelling of the feet. Three months before she was seen she developed paraesthesiae and numbness in the feet and some weeks later similar symptoms in the hands. Dietetic inquiry did not suggest any deficiency of vitamins in the diet, nor was there any history of an excessive intake of alcohol. The patient had been taking thalidomide, in a nightly dose of 200 mg., for some months before the onset of symptoms.

Her nutrition appeared satisfactory; the blood-pressure was 160/90 mm. Hg and there was slight oedema of the ankles, but no elevation of the jugular venous pressure; the general examination was otherwise normal. In the motor system the only abnormalities found were sluggish knee-jerks and absent ankle-jerks; the plantar responses were flexor. There was a patchy impairment in the appreciation of light touch and pin-prick in both legs below the level of the mid-calf, but no sensory disturbance could be detected in the arms.

Investigations.—The haemoglobin was 14.7 g./100 ml., and the red blood cells and their indices were normal. A fractional test meal showed the presence of normal quantities of free